

Molecular Basis of Restenosis and Drug-Eluting Stents

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Resolution of restenosis probably requires both creation of the largest possible residual lumen and substantial inhibition of intimal hyperplasia.

—J.S. Forrester and coworkers¹

Dr Forrester's prediction that resolution of restenosis would require the translational merging of molecular mechanisms of proliferation with local scaffolding and drug-delivery devices appears to have been remarkably prescient. The application of drug-eluting stent (DES) technology to improve clinical outcomes after percutaneous coronary intervention (PCI) represents one of the greatest success stories in cardiology. This review highlights the molecular basis of restenosis and DES for the clinical and interventional cardiologist and vascular biologist.

Restenosis: Definitions and Mechanisms

Restenosis is the arterial wall's healing response to mechanical injury and comprises 2 main processes—neointimal hyperplasia (ie, smooth muscle migration/proliferation, extracellular matrix deposition) and vessel remodeling. Primarily on the basis of observations from animal studies, Forrester and coworkers¹ proposed a paradigm for neointimal hyperplasia as a general wound-healing response. Platelet aggregation, inflammatory cell infiltration, release of growth factors, medial smooth muscle cell (SMC) modulation and proliferation, proteoglycan deposition, and extracellular matrix remodeling were identified as the major milestones in the temporal sequence of this response. This view of the neointimal hyperplasia process was modified subsequently by Libby and colleagues² to reconcile certain important clinical features—namely, that thrombosis, often invoked as a cause of SMC proliferation, wanes before intimal thickening peaks and that antithrombotic therapy failed to eliminate restenosis. In this cascade model, a special case was made for the centrality of inflammation, and it was proposed that autocrine or paracrine mediators (eg, interleukin-1 [IL-1] and tumor necrosis factor), the expressions of which are triggered by vascular injury, contribute to deranged SMC behavior during restenosis.

The molecular mechanisms of the arterial remodeling are less well understood. The term *remodeling* has been applied largely to describe either vascular shrinkage or enlargement. The definition proposed by Schwartz et al,³ in which remodel-

ing is characterized in a continuous spectrum by any change in vascular dimension, may better describe this compensatory phenomenon. Studies that used intravascular ultrasound provided the first evidence of the key role of negative remodeling (vessel shrinkage) on lumen deterioration in nonstented coronary arteries.^{4,5} Adventitial myofibroblasts, which are capable of collagen synthesis and tissue contraction as seen in wound healing,^{6,7} may play an important role in negative vessel remodeling observed in restenosis after balloon angioplasty. The association between enhanced inflammatory response and vessel enlargement, as observed in an experimental study, represents a potential protective effect against negative vessel remodeling.^{7,8} Nevertheless, remodeling is virtually absent after stenting as observed by volumetric intravascular ultrasound (IVUS).^{9,10} The superior outcomes of bare-metal stents compared with angioplasty result mainly from the scaffold property of these metallic prostheses, which prevents vessel shrinkage (elastic recoil and negative remodeling), despite inducing an enhanced neointimal hyperplasia response. The molecular basis of this enhanced proliferative response has been the focus of pharmacological prevention of restenosis and led to the ultimate development of DES.

An Integrated View of the Pathophysiology of Restenosis

An integrated view of the molecular and cellular events of in-stent restenosis has been proposed by Welt and Rogers (Figure 1).¹¹ A series of events are initiated immediately after balloon inflation or stent deployment. The initial consequences immediately after stent placement are deendothelialization, crush of the plaque (often with dissection into the tunica media and occasionally adventitia), and stretch of the entire artery. A layer of platelets and fibrin is then deposited at the injured site. Activated platelets on the surface expressing adhesion molecules such as P-selectin attach to circulating leukocytes via platelet receptors such as P-selectin glycoprotein (GP) ligand and begin a process of rolling along the injured surface. Leukocytes then bind tightly to the surface through the leukocyte integrin (ie, Mac-1) class of adhesion molecules via direct attachment to platelet receptors such as GP Ib α and through cross-linking with fibrinogen to the GP IIb/IIIa receptor. Migration of leukocytes across the platelet-fibrin layer and diapedesis into the tissue is driven by

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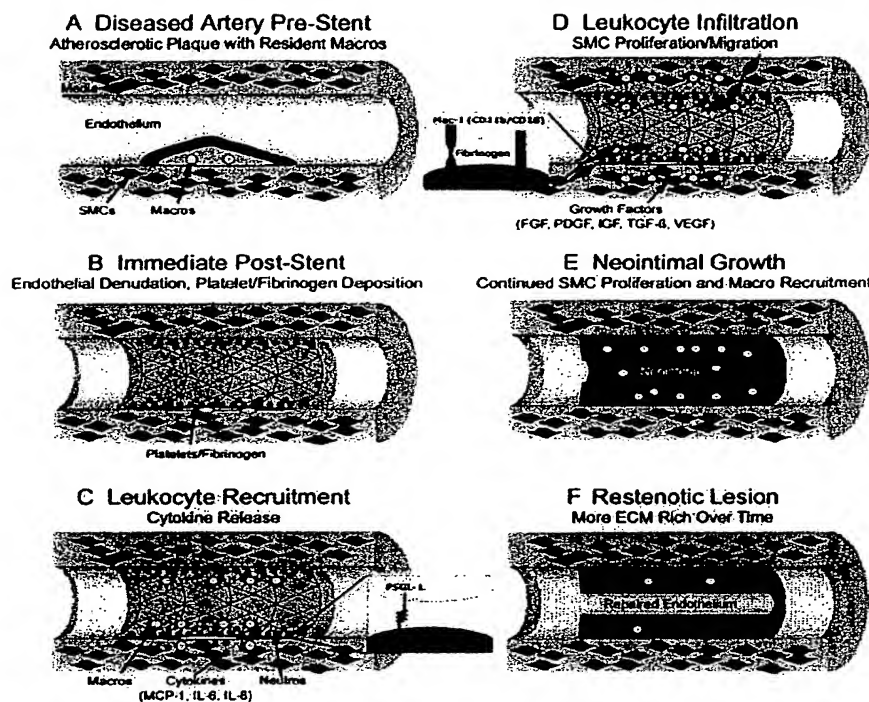


Figure 1. Schematic of an integrated cascade of restenosis. A, Atherosclerotic vessel before intervention. B, Immediate result of stent placement with endothelial denudation and platelet/fibrinogen deposition. C and D, Leukocyte recruitment, infiltration, and SMC proliferation and migration in the days after injury. E, Neointimal thickening in the weeks after injury, with continued SMC proliferation and monocyte recruitment. F, Long-term (weeks to months) change from a predominantly cellular to a less cellular and more ECM-rich plaque. Reprinted with permission from *Arterioscler Thromb Vasc Biol*.¹¹

chemical gradients of chemokines released from SMCs and resident macrophages.

Next is a granulation or cellular proliferation phase. Growth factors are subsequently released from platelets, leukocytes, and SMCs, which stimulate migration of SMCs from the media into the neointima. The resultant neointima consists of SMCs, extracellular matrix, and macrophages recruited over several weeks. Cellular division takes place in this phase, which appears to be essential for the subsequent development of restenosis.

Over longer time periods, the artery enters a phase of remodeling involving extracellular matrix (ECM) protein degradation and resynthesis. Accompanying this phase is a shift to fewer cellular elements and greater production of ECM. ECM is composed of various collagen subtypes and proteoglycans¹² and constitutes the major component of the mature restenotic plaque.¹³ In the balloon-angioplastied artery, reorganization of the ECM,¹⁴ replacing hydrated molecules by collagen, may lead to shrinkage of the entire artery and negative remodeling. In the stented artery, this phase has less impact because of minimal negative remodeling, although constituents of ECM such as hyaluronan, fibronectin, osteopontin and vitronectin also facilitate SMC migration.^{15,16} In both balloon-angioplastied and stented arteries, reendothelialization of at least part of the injured vessel surface may occur.

Animal Model and Human Evidence for the Role of Inflammation in Restenosis

Experimental and clinical data indicate that leukocytes may be central to intimal growth after mechanical arterial injury. In animal models of vascular injury, leukocytes are recruited

as a precursor to intimal thickening.^{17,18} In animal models in which a stent is deployed to produce deep vessel wall trauma, a brisk early inflammatory response is induced with abundant surface-adherent neutrophils and monocytes.¹⁸ Days and weeks later, macrophages accumulate within the developing neointima and are observed clustering around stent struts. The number of vessel wall monocytes/macrophages is positively correlated with the neointimal area, suggesting a possible causal role for monocytes in restenosis. We and others have shown that blockade of early monocyte recruitment results in reduced late neointimal thickening.^{19–21} Leukocytes likely modulate vascular repair through multiple mechanisms. Inflammatory cells may contribute to neointimal thickening because of their direct bulk within the intima,²² generation of injurious reactive oxygen intermediates,²³ elaboration of growth and chemotactic factors,²⁴ or production of enzymes (eg, matrix metalloproteinases, cathepsin S) capable of degrading extracellular constituents and thereby facilitating cell migration.^{25,26}

Quantitative immunohistochemical analysis of directional coronary atherectomy specimens from humans has shown that the number of macrophages present in the tissue at the time of angioplasty predicts future restenosis.²² Farb et al²⁷ reported findings from pathological studies of 116 stents from 56 patients after PCI. They found a strong link between the extent of medial damage, inflammation, and neointimal thickness. They reported a late occurrence of peri-strut neoangiogenesis, which was strongly correlated with inflammation. The causal relationship between new blood vessels and clinical restenosis could not be firmly established.

Systemic markers of inflammation also appear to be predictive of restenosis after balloon angioplasty. Stenting of

patients with stable angina and low C-reactive protein levels at baseline is associated with a transient rise in C-reactive protein that returns to baseline within 48 to 72 hours.²⁸ Sustained elevations of C-reactive protein are associated with an increased risk of clinical and angiographic restenosis. Using flow cytometry, several groups have reported independently that balloon angioplasty and stenting are associated with upregulation of neutrophil CD11b that is positively correlated with clinical restenosis and late lumen loss,^{29–31} and that cell activation occurred across the mechanically injured vessel.

Molecular Mechanisms of Inflammation

Adhesive interactions between vascular cells play important roles in orchestrating the inflammatory response. Recruitment of circulating leukocytes to vascular endothelium requires multistep adhesive and signaling events (including selectin-mediated attachment and rolling, leukocyte activation, and integrin-mediated firm adhesion and diapedesis) that result in the infiltration of inflammatory cells into the blood vessel wall.³² Firm attachment is mediated by members of the β_2 integrin family, LFA-1 ($\alpha_L\beta_2$, CD11a/CD18), Mac-1 ($\alpha_M\beta_2$, CD11b/CD18), and p150,95 ($\alpha_M\beta_2$, CD11c/CD18), which bind to endothelial counterligands (eg, intercellular adhesion molecule-1),³³ to endothelial-associated ECM proteins (eg, fibrinogen),³⁴ or to glycosaminoglycans.³⁵

Leukocyte recruitment and infiltration also occur at sites of vascular injury where the lining endothelial cells have been denuded and platelets and fibrin have been deposited. The initial tethering and rolling of leukocytes on platelet P-selectin³⁶ are followed by their firm adhesion and trans-platelet migration, processes that are dependent on leukocyte Mac-1³⁷ and platelet GP Ib α .³⁸ The precise cellular and molecular mechanisms of inflammation after arterial injury are highly dependent on the specific type of injury (ie, stent versus balloon and mechanical versus atherogenesis). For example, experimental stent deployment in animal arteries causes sustained elevation of monocyte chemoattractant protein-1 (MCP-1) after injury (\approx 14 days) compared with balloon-injured arteries ($<$ 24 hours).³⁹ Correspondingly, antibody-mediated blockade of CCR2, a primary leukocyte receptor for MCP-1, markedly diminished neointimal thickening after stent-induced but not balloon-induced injury in nonhuman primates.⁴⁰ In contrast to targeting Mac-1, which reduces neointimal thickening after experimental angioplasty^{19,40,41} but does not attenuate atherogenesis,⁴² targeting MCP-1 also appears to benefit arteries affected by either mechanical injury or atherogenesis. Mice genetically deficient in MCP-1⁴³ or CCR2⁴⁴ demonstrated significant reductions in aortic lipid content, monocyte accumulation, and atherosclerotic lesion development, as well as neointimal thickening after experimental angioplasty.⁴⁵

Experimental observations support a causal relationship between inflammation and experimental restenosis. Antibody-mediated blockade¹⁹ or selective absence of Mac-1⁴¹ diminished leukocyte accumulation and limited neointimal thickening after experimental angioplasty or stent implantation. Targeting earlier, selectin-mediated interactions between platelets and leukocytes also markedly reduces

leukocyte recruitment and neointimal thickening in a variety of animal models.⁴⁶ Blockade of the MCP-1 receptor CCR2 has been shown to reduce neointimal thickening within stented arterial segments. Interestingly, MCP-1 is upregulated after PCI in humans, and MCP-1 levels correlate with risk for restenosis.⁴⁷

Antiinflammatory Approaches to Prevent Clinical Restenosis

On the basis of overwhelming experimental and clinical evidence that inflammation drives restenosis, several investigators are pursuing clinical programs using systemic anti-inflammatory therapies, including liposome-encapsulated bisphosphonates,⁴⁸ prednisone,⁴⁹ anti-CD18 or anti-CCR2 blockade,⁴⁰ and peroxisome proliferators-activated receptor- γ activator rosiglitazone.

Corticosteroids have long been shown to reduce the influx of mononuclear cells, to inhibit monocyte and macrophage function, and to influence SMC proliferation⁵⁰; however, clinical trials with systemic steroid therapy have shown mixed results.^{49,51} Stents eluting steroid agents such as methylprednisolone (300 mg) were used in a porcine model and showed a reduction in neointimal proliferation as compared with a severe intimal hyperplasia promoted by the stent coated with polymer.⁵² The clinical correlate of this experiment was the STRIDE (Study of Anti-Restenosis with BiodivYsio Dexamethasone-Eluting Stent) study conducted in Europe. Restenosis ($>$ 50% diameter stenosis at follow-up) was 13.3% and late loss (the difference between the minimal luminal diameter [MLD] immediately after the procedure and the MLD at follow-up) was 0.45 mm (I. De Scheerder, MD, unpublished data, 2002). The inadequate release profile of the pharmacological agent, eluted almost completely in the first 24 hours after deployment, likely influenced the clinical effect of the drug. Tranilast, *N*-(3,4-dimethoxycinnamoyl) anthranilic acid, also has been shown to inhibit proliferation and migration of vascular SMCs in experimental models. A large multicenter trial failed to show antirestenosis effects of this agent administered systemically.⁵³ Initial experiments with the biodegradable Igaki-Tamai stent loaded with 184 μ g of tranilast per stent have been initiated.

Human Evidence for the Role of Cellular Proliferation in Restenosis

Experimental studies have suggested that SMC proliferation is critical to neointimal formation after mechanical injury, including wire-, balloon-, and stent-induced injury. Using proliferation markers, such as proliferating nuclear cell antigen (PCNA) and incorporation of BrdU, these studies have observed peak proliferation rates of up to 10% to 20% of total medial cells 5 to 7 days after injury.^{19,41,54} Human pathological data with regard to this issue are limited and controversial. Kearney and coworkers⁵⁵ retrieved in-stent restenotic tissue by directional atherectomy from 10 patients after percutaneous revascularization of peripheral artery disease. Cellular proliferation was evaluated via the use of antibodies to PCNA, cyclin E, and cdk2. Directional atherectomy tissue contained areas composed predominantly of SMCs. Evidence of ongoing SMC proliferative activity also was documented:

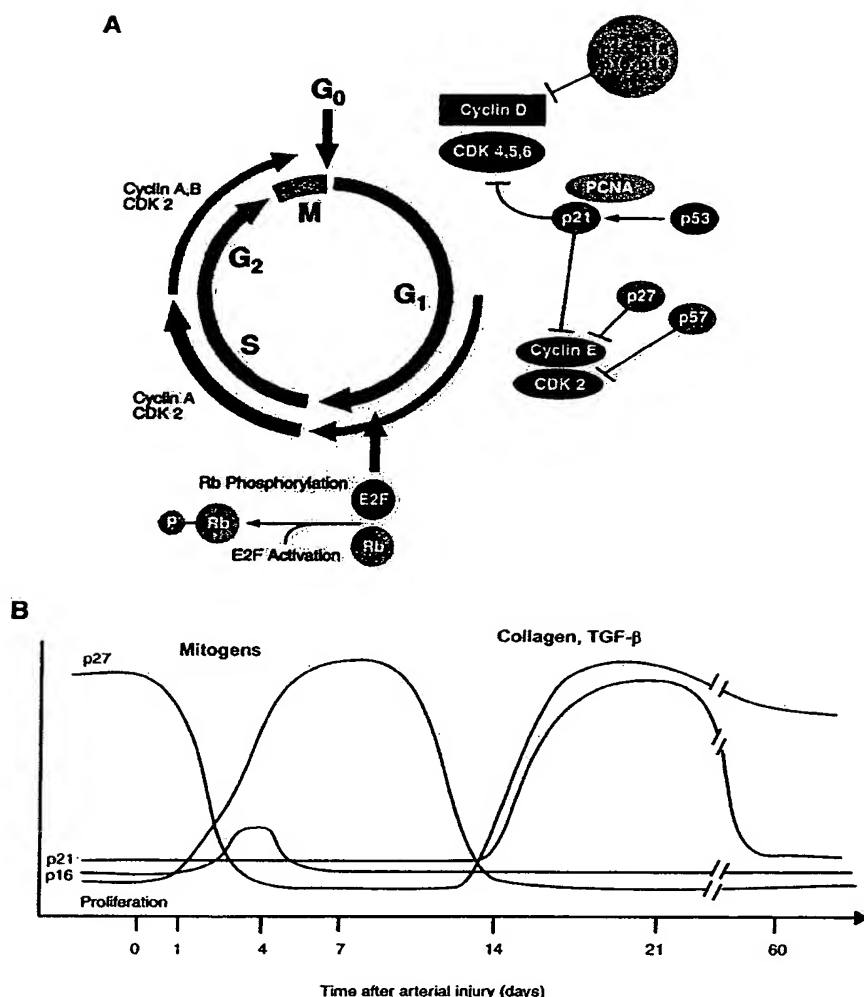


Figure 2. A, Regulation of the cell cycle. Progression through G₁ phase of the cell cycle occurs by the assembly and phosphorylation of cyclin and CDKs. The CKIs act as brakes to halt the cyclin and arrest cycles. PCNA indicates proliferating cell nuclear antigen; Rb, retinoblastoma; P, phosphorylated. Reprinted with permission from *Circulation*.⁷⁴ B, Schematic representation of the temporal role of CKIs (p27^{Kip1}, p21^{Cip1}, and p16^{INK4}), transforming growth factor-β, and collagen in SMC proliferation during arterial repair in porcine arteries.

24.6±2.3% of SMCs were PCNA positive, 24.8±3.1% were cyclin E positive, and 22.5±2.2% were cdk2 positive. In contrast, O'Brien and colleagues estimated that the maximum percentage of cells that were replicating was <1.2%, as shown by the expression of H3 mRNA⁵⁶; however, high levels of focal replication also were observed, with up to 6.6% replicating cells. There is also evidence that cells of monocyte/macrophage lineage (HAM-56 positive) proliferate within human in-stent restenotic tissue.¹¹ The discrepancy between the levels of replication in animals and humans is likely secondary to the fact that animal studies examine proliferation at early time points (<1 week) when proliferation peaks, whereas human studies assess proliferation late (>3 months) when proliferation wanes. Nevertheless, the effectiveness of rapid-release sirolimus (>90% eluted in 30 days) in reducing late loss after clinical stenting⁵⁷ supports the biological relevance of early cellular proliferation even in humans. Taken together these observations support the notion that neointimal hyperplasia results from vascular cell (ie, SMC and monocyte/macrophage) proliferation and provide the basis for antirestenosis strategies targeting cell cycle division early after stent implantation.

Cell Cycle and Restenosis

Under normal conditions, SMCs are quiescent and exhibit low levels of proliferative activity. Mechanical injury or growth factors trigger the SMC progress through the G₁/S transition of the cell cycle^{58,59} (Figure 2). The different phases of the cell cycle of eukaryotic cells are regulated by a series of protein complexes composed of cyclins (D, E, A, B), cyclin-dependent kinases (CDKs; CDK4, CDK2, p34^{cdc2}) and their cyclin-dependent inhibitors (CKIs; p27^{Kip1}, p70, p16^{INK4}).^{60–62} The function of CKIs is regulated by changes in their concentration as well as in their localization in the cell. The concentration of p27^{Kip1} is controlled predominantly by the ubiquitin-proteasome pathway.⁶³ The CKIs have distinct temporal and spatial patterns of expression in normal, injured, and diseased arteries (Figure 2).⁵⁸ p27^{Kip1} is downregulated after arterial injury when cell proliferation increases. p21^{Cip1} is not observed in normal arteries but is upregulated along with p27^{Kip1} in later phases of arterial healing response and is associated with a significant decline in cell proliferation and an increase in procollagen and transforming growth factor-β synthesis.⁵⁸ These findings suggest that p27^{Kip1} and p21^{Cip1} are endogenous regulators of G₁ transit in vascular SMCs and

Summary of Drug-Eluting Stent Clinical Data

Study (n)	Randomized	Drug/Agent	Stent	In-Stent Late Loss* (Time of Follow-Up)	MACE Rates* (Time of Follow-Up)
First-In-Man (45)	No	Sirolimus	BX Velocity	FR=0.41 mm (4 y) SR=0.09 mm (4 y)	2% (all, 12 mo) FR=20% (4 y) SR=7% (4 y)
RAVEL (240)	Yes	Sirolimus	BX Velocity	-0.01 (6 mo)	5.8% (12 mo)
SIRIUS (1058)	Yes	Sirolimus	BX Velocity	0.17 mm (8 mo)	8.3% (12 mo)
TAXUS II (536)	Yes	Paclitaxel	NIR	SR=0.31 mm (6 mo) MR=0.3 mm (6 mo)	SR=10.9% (12 mo) MR=9.9% (12 mo)
TAXUS IV (1314)	Yes	Paclitaxel	Express 2	SR=0.39 mm (9 mo)	SR=10.6% (12 mo)
TAXUS VI (446)	Yes	Paclitaxel	Express 2	MR=0.39 mm (9 mo)	MR=16.4% (9 mo)
FUTURE I (42 nondiabetic)	Yes	Everolimus	Challenge	0.11 mm (6 mo)	7.7% (6 mo)
FUTURE II (64)	Yes	Everolimus	Challenge	0.12 mm (6 mo)	4.8% (6 mo)
ENDEAVOR I (100)	No	ABT-578	Driver	0.58 mm (12 mo)	2% (12 mo)
IMPACT (150)	Yes	Mycophenolic acid	Duraflex	FR=1.04 mm (6 mo) SR=0.95 mm (6 mo)	FR=12% (6 mo) SR=16.1% (6 mo)
PRESENT (22)	No	Tacrolimus	Ceramic-Coated Flex	0.81 mm (6 mo)	13.6% (6 mo)
EASTER (30)	No	Estradiol	ByodivYsio	0.54 mm (6 mo)	3.3% (1 y)
NOBLESSE (45)	No	Oxygen free radical scavenger	Genic Stent	0.69 mm (4 mo)	6.7% (4 mo)
STEALTH I (100)	No	Biolimus A9	Challenge	N/A	N/A
HEALING II (60)	No	Anti-CD34 antibodies	R Stent	N/A	N/A

MACE indicates major adverse cardiac event (eg, death, MI, repeat revascularization); FR, fast release; SR, slow release, and N/A, not available.

*Data provided for the active treatment groups.

inhibit cell proliferation after arterial injury. $p27^{Kip1}$ and $p21^{Cip1}$ bind and alter the activities of cyclin D-, cyclin E-, and cyclin A-dependent kinases (CDK2) in quiescent cells, leading to the failure of G_1/S transition and cell cycle arrest.^{64,65} Overexpression of $p27^{Kip1}$ results in cell cycle arrest in the G_1 phase.⁶⁶ Gene transfer of $p27^{Kip1}$ or $p21^{Cip1}$ into balloon-injured arteries produces a significant reduction in SMC proliferation and neointimal thickening.^{58,67,68} Conversely, inhibition of $p27^{Kip1}$ increases the number of cells in S phase.⁶⁹ $p27^{Kip1}$ -deficient mice develop hyperplasia in multiple organs, including endocrine tissues, thymus, and spleen.⁷⁰⁻⁷² Importantly, deficiency of $p27^{Kip1}$ has a prominent vascular phenotype with markedly increased neointimal thickening and inflammatory cell accumulation after mechanical arterial injury.⁵⁴

The level of $p27^{Kip1}$ is also regulated by constituents of the ECM. Mature collagen (polymerized type I collagen) suppresses $p70^{S6k}$ and has been shown to increase the levels of $p27^{Kip1}$, whereas monomeric collagen, which is present during degradation of ECM in the synthesis phase of restenosis, downregulates $p27^{Kip1}$. In addition, SMC migration appears to be regulated by the cell cycle.⁷³ SMCs in G_1 but not in later phases of the cell cycle have the ability to migrate on mitogenic stimulus, but the upregulation of $p27^{Kip1}$ inhibits cellular migration.^{74,75}

The cell cycle is a common hub of the different phases of the restenosis process. The unprecedented clinical successes of recent antirestenosis approaches targeting cellular division pathways illustrate its central role in the formation of neointimal hyperplasia. Currently available DES technologies deliver high concentrations of immunosuppressive or antitumor

agents into the vessel wall. The specific molecular and cellular effects of these agents are discussed in the sections below. Clinical data on DES technologies were recently reviewed in this journal and are summarized in the Table.⁷⁶

Rapamycin

Rapamycin (sirolimus) is a natural macrocyclic lactone with potent immunosuppressive properties. The drug was isolated from *Streptomyces hygroscopicus* in the mid 1970s and approved by the Food and Drug Administration for the prophylaxis of renal transplant rejection in 1999 (Figure 3A). Other rapamycin analogs, such as everolimus, ABT-578, biolimus-A9, and temsirolimus, have been developed recently, and clinical investigations testing the safety and antirestenotic effects of these agents are under way.

Mechanism of Action

Rapamycin is actually a pro-drug that binds to specific cytosolic proteins. The mechanism of action of sirolimus is distinct from other immunosuppressive agents that act solely by inhibiting DNA synthesis. Sirolimus binds to the immunophilin FK506-binding protein 12 (FKBP12), which is upregulated in human neointimal SMCs.^{77,78} FKBP12-independent effects of rapamycin remain unknown. The FKBP12/rapamycin complex binds to a specific cell cycle-regulatory protein, the mTOR (mammalian target of rapamycin), and inhibits its activation. TOR is a member of the PI3K-related protein kinase (PIKK) family and is composed of up to 20 tandemly repeated motifs. PIKKs are involved with critical steps of the cell cycle, including checkpoints that govern DNA damage and repair.⁷⁹ The C-terminus of mTOR

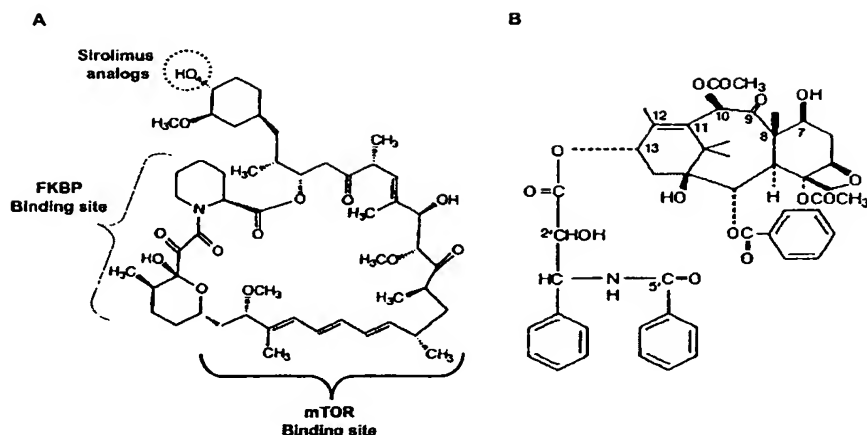


Figure 3. A, Chemical structure of sirolimus showing the binding sites of FKBP, mTOR, and radical group that is replaced in other rapamycin analogs (everolimus: $\text{OCH}_2\text{CH}_2\text{OH}$; ABT-578: tetrazole; biolimus A9: $\text{OCH}_2\text{CH}_2\text{OR}$). Courtesy of Dr Robert Falotico, Cordis Johnson & Johnson, and used with permission. B, Structure of paclitaxel. Reprinted with permission from *N Engl J Med*.⁹⁵ Copyright © 1995, Massachusetts Medical Society. All rights reserved.

contains the FKBP12/rapamycin-binding domain. TOR binding to rapamycin is mediated by a 150-kDa peptide called raptor (regulatory-associated protein of mTOR).⁸⁰ Another protein named GbetaL has been shown to stabilize the interaction between raptor and mTOR. Recently, mTOR also has been shown to be part of a distinct complex independent of rapamycin.⁸¹

The precise downstream molecular effects of the inhibition of mTOR by rapamycin are not completely understood. mTOR is involved with a crucial event of the cell cycle, the transition between the G_1 and S phase, in which DNA replication occurs, thus leading to irreversible cellular commitment toward division (Figure 4). Thus, rapamycin has been shown to have a cytostatic effect and to induce cell-cycle arrest in late G_1 phase.^{78,82} A known effect of rapamycin is the inhibition of serine/tyrosine kinase $p70^{\text{S6k}}$ and upregulation of the $p27^{\text{Kip1}}$ (discussed above)⁸³ (Figure 4); however, at higher doses, rapamycin may inhibit cellular migration through a mechanism that is independent of $p27^{\text{Kip1}}$.⁷⁵

Rapamycin has been shown to inhibit all phases of the restenosis cascade. Suzuki and colleagues tested sirolimus-

eluting stents in porcine restenosis models.⁸⁴ Inflammation was markedly reduced in the treatment group, which paralleled the inhibition in neointimal hyperplasia formation.⁸⁴ Reendothelialization occurred at a similar degree in both DES and bare-metal stent groups. Reendothelialization after sirolimus-eluting stents also has been confirmed in human coronary arteries.⁸⁵ Rapamycin also has been shown to inhibit total protein and collagen synthesis involved in ECM formation. Furthermore, rapamycin inhibits SMC migration^{75,86} and promotes a contractile rather than a proliferative phenotype.⁸⁷ As a result of these multiple mechanisms of action, sirolimus-eluting stents have been shown to reduce neointimal thickening as compared with both bare-metal and polymer-coated stents in various animal models and clinical trials.^{57,86,88–92}

The most sensitive cell lines respond to rapamycin at the nanomolar level (50% inhibitory concentration [IC50] <2 nmol/L). Rapamycin is effective over a range of doses (18 to 1200 $\mu\text{g}/18\text{-mm}$ stent) in animal models. The current dose applied onto the stents is 140 $\mu\text{g}/\text{cm}^2$ (180 $\mu\text{g}/18\text{-mm}$ stent), whereas 1200 μg of rapamycin per 18-mm stent did not reach toxic levels. This broad toxic-therapeutic window is critical

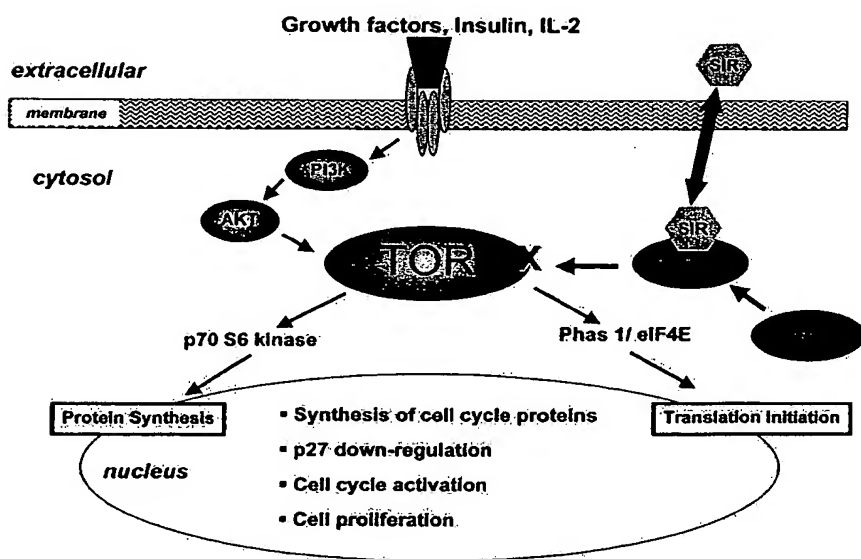


Figure 4. Upstream and downstream pathways of mTOR. Membrane receptors activated by various growth factors (ie, insulin, IL-2). G-protein couple receptors cause activation of phosphatidylinositol-3 kinase, which catalyzes the conversion of phosphatidylinositol-4,5-bisphosphate (PIP2) to phosphatidylinositol-3,4,5-triphosphate (PIP3). PIP3 binds to a serine-threonine kinase, Akt, which affects the phosphorylation states of mTOR. Downstream, mTOR phosphorylates the phosphorylated heat-stable and acid-stable protein (Phas-1), which is a translational repressor that binds to eIF4E. This process releases the eukaryotic initiation factor eIF4F for initiation of protein translation. mTOR also stimulates the $p70^{\text{S6k}}$ mitogen-stimulated kinase. These processes are blocked by the FKBP12-sirolimus (SIR) complex.

because these devices are implanted in diseased human coronary arteries, which are highly heterogeneous in composition and asymmetric. Of note, the first series of patients treated with sirolimus-eluting stents have recently completed 5-year clinical and angiographic follow-up without evidence for adverse reactions associated with the device.

Taxanes

Paclitaxel (Taxol; Bristol-Myers Squibb) was isolated from the bark of the Western yew tree in 1971.⁹³ Paclitaxel is a diterpenoid compound that contains a complex 8-member taxane ring as its nucleus (Figure 3B). The side chain linked to the taxane ring at carbon 13 is essential for its antitumor activity. Modification of the side chain has led to identification of the more potent analog, docetaxel (Taxotere; Aventis Pharmaceuticals), which shares the same spectrum of clinical activity as paclitaxel but differs in its spectrum of toxicity. Originally purified as the parent molecule from yew bark, paclitaxel can now be obtained for commercial purposes by semisynthesis from 10-desacetylbaicatin, a precursor found in yew leaves. It also has been successfully synthesized⁹⁴ in a complex series of reactions. Paclitaxel has limited solubility and must be administered in a vehicle of 50% ethanol and 50% polyethoxylated castor, a formulation that is likely responsible for a high rate of hypersensitivity reactions.

Paclitaxel and docetaxel exhibit unique pharmacological action as inhibitors of mitosis, differing from the vinca alkaloids and colchicine derivatives in that they bind to a different site on β -tubulin and promote rather than inhibit microtubule formation. The drugs play a central role in the therapy of ovarian, breast, lung, esophageal, bladder, and head and neck cancers.⁹⁵

Mechanism of Action

Interest in paclitaxel was stimulated by the finding that the drug possessed the unique ability to promote microtubule formation at cold temperatures and in the absence of guanosine 5'-triphosphate.^{96,97} Microtubules form the mitotic spindle during cell division and are important in other cell functions, including maintenance of cell shape, motility, and intracellular transport.⁹⁵ Paclitaxel binds specifically to the β -tubulin subunit of microtubules and appears to antagonize the disassembly of this key cytoskeletal protein, with the result that bundles of microtubules and aberrant structures derived from microtubules accumulate in the mitotic phase of the cell cycle. Arrest in mitosis (G_2/M phase) follows. Cell killing is dependent on both drug concentrations and duration of cell exposure. Paclitaxel enhances the cytotoxic effects of ionizing radiation in vitro, possibly by inducing arrest in the premitotic G_2 and mitotic phases of the cell cycle, which are the most radiosensitive phases.⁹⁵ Drugs that block the progression of cells through DNA synthesis (eg, sirolimus) and into mitosis may antagonize the toxic effects of taxanes. This may have significant implications for the clinical scenario of overlapping a sirolimus-eluting stent with a paclitaxel-eluting stent.

Paclitaxel also has distinct cell cycle-independent effects. Paclitaxel is capable of influencing cellular spreading and migration as a consequence of its effect on microtubule

function.^{98,99} The avidity of cell adhesion molecules, such as integrins, is regulated by cytoskeletal constraints, which keep integrin in an inactive state. Releasing these constraints results in increased lateral mobility and clustering of integrins, which effectively activate adhesion. Depolymerization of microtubules by colchicines or nocodazole and stabilization of microtubules by paclitaxel increase integrin mobility and activate adhesion.⁹⁹ In SMCs, these effects lead to a pronounced inhibition of SMC migration in vitro and in vivo.^{98,100} A series of studies have shown that paclitaxel and lipopolysaccharide induce strikingly similar responses in murine macrophages.^{101,102} Paclitaxel provides a second signal for murine macrophage tumoricidal activity via L-arginine-dependent nitric oxide (NO) synthesis^{101,102} that appears to require paclitaxel binding to CD18¹⁰³ and protein kinase C.¹⁰² Paclitaxel also has immunomodulatory properties. Through its capacity to induce IL-12 production, paclitaxel may contribute to the correction of tumor-induced immune dysfunction.^{104,105}

Resistance

In cultured tumor cells, resistance to taxanes is associated in some lines with increased expression of the *mdr*-1 gene and its product, the P-glycoprotein; other resistant cells have β -tubulin mutations, and these latter cells may display heightened sensitivity to vinca alkaloids.¹⁰⁶ Other cell lines display an increase in surviving, an antiapoptotic factor,¹⁰⁷ or aurora kinase, an enzyme that promotes the completion of mitosis.¹⁰⁸ The basis of clinical drug resistance is not known.

Therapeutic Uses

Paclitaxel and docetaxel have become central components of regimens for treating metastatic ovarian, breast, lung, and head and neck cancers.^{109,110} Docetaxel has significant activity with estramustine for the treatment of hormone-refractory prostate cancer. Both drugs are used in either once-weekly or once-every-3-week regimens, with comparable response rates and somewhat different patterns of toxicity. The optimal schedule of taxane administration, alone or in combination with other drugs, is still under evaluation.

Unlike other antimitotic agents, paclitaxel shifts the cytoskeleton equilibrium toward assembly, leading to reduced vascular cell proliferation, migration, and signal transduction.⁹⁸ Stents eluting paclitaxel (200 μ g per stent) reduced neointimal and medial cell proliferation at all time points (7, 28, 56, and 180 days) when placed in porcine coronary arteries¹¹¹; however, arteries treated with paclitaxel showed incomplete healing, late persistence of a large number of macrophages, and fibrin deposition. Similar findings were observed with a stent platform coated with cross-linked biodegradable polymer (chondroitin sulfate and gelatin) in rabbit iliac arteries.¹¹² These studies indicated the need for a tightly controlled drug release of paclitaxel because of its narrow toxic-therapeutic window and hydrophobic properties.

Early clinical studies of stents coated with 2 to 3 μ g/mm² of paclitaxel without a polymer coating showed suboptimal antirestenosis effects.¹¹³⁻¹¹⁵ On the other hand, stents coated with a poly(lactide-co- Σ -caprolactone) to control the release of the drug were effective in reducing late loss and restenosis

in a series of clinical trials, the TAXUS studies.^{116–118} Most of these studies tested stents coated with 85 μg of paclitaxel ($1.0 \mu\text{g}/\text{mm}^2$) with an initial burst phase over the first 48 hours after implantation followed by a slow-release phase for 10 days (ie, slow-release formulation), but $>80\%$ of the drug remains unreleased from the polymer coating. The moderate-release Taxus stent (Boston Scientific) delivers an 8-fold higher dose of Taxol in the first 10 days.

Local Drug Pharmacokinetics

The biological effects of any pharmacological agent delivered locally are influenced by local transport forces, which are related to the properties of the target tissue.^{119,120} The highly heterogeneous composition of the arterial wall¹²¹ and its asymmetric geometrical organization represents a challenge for most agents applied in DES technologies. The ideal compound for intramural delivery should contain hydrophobic elements to ensure high local concentrations as well as hydrophilic properties to allow homogeneous drug diffusion. Altered transport of these agents through the vessel wall may lead to both toxic levels in areas where the drug may accumulate (ie, surrounding the stent struts) and nontherapeutic levels in remote regions away from the drug reservoir (ie, adventitial tissue). In bovine internal carotid segments, tissue-loading profiles for rapamycin and paclitaxel were similar. Both drugs bind to the artery at 30 to 40 times bulk concentration; however, these drugs showed markedly different profiles of transmural distribution, with rapamycin distributing evenly through the artery, whereas paclitaxel remains primarily in the subintimal space.¹²²

Clinical Aspects of Restenosis after DES

Gruntzig and coworkers¹²³ observed that most clinical ischemic events related to vessel renarrowing occurred between 3 and 9 months after balloon angioplasty. This seminal observation illustrates the delay between the biological process and symptomatic presentation of restenosis, which results in a 70% increase in the incidence of target lesion revascularization between 6 and 12 months after the procedure.¹²⁴ The time frame to develop clinical signs of restenosis after DES may be further extended because antiproliferative agents may delay the biological response to injury. Indeed, intimal proliferation after brachytherapy seems to have a different time course¹²⁵ and pathological characteristics, as IVUS already elicited its echolucent appearance, dubbed “black hole.”¹²⁶

In routine practice, noninvasive assessment of restenosis is an appropriate approach; however, objective criteria of restenosis are required in clinical trials testing the antiproliferative effects of a given therapy. The use of continuous angiographic criteria of lumen deterioration, namely late lumen loss, more closely reflects the magnitude of the intimal hyperplasia response after stenting.^{127,128} Late loss represents an angiographic surrogate for neointimal proliferation within the stented segment because the remodeling component of restenosis is abolished. In adjacent nonstented segments (ie, in-segment and edge segments), the value of late loss to define vessel wall biological response to injury is limited by multiple confounding factors that influence luminal dimensions, such as elastic recoil, vessel spasm, and remodeling.

Late loss has been classically calculated as MLD immediately after the procedure minus MLD at follow-up without consideration of the location of the MLD. Angiographic measurements, however, are highly dependent on the site of the measurements and location of MLD. A mismatch between the location of the MLD between immediately after the procedure and follow-up, which occurs frequently,¹²⁹ affects the calculation of the true luminal deterioration, and consequently the proper assessment of the neointimal proliferation.

A customized angiographic methodology, which includes individual subsegmental (5 mm per segment) quantifications, has been proposed to calculate “true” late loss, which compares MLD between postprocedure and follow-up measurements at matched locations (M.A.C., unpublished data, 2004). This methodology allows the assessment of the vascular response (ie, late loss) at individual sites along the entire target segment. The variations in drug distribution, degree of injury, and tissue composition along the target vessel wall provide substrates for heterogeneous local vessel wall responses, which have been observed after balloon angioplasty.¹³⁰ Indeed, preliminary data from our core laboratory showed a mismatch between the sites of MLD in $>50\%$ of the assessments in patients with diabetes treated with either bare-metal or sirolimus-eluting stents. The relocation-of-MLD phenomenon resulted in a 0.26-mm difference between late loss measurements (unmatched versus matched MLD sites).

Although late loss represents the best angiographic surrogate of the biological arterial wall response in stented segments, its value as a clinically relevant end point has been questioned. The correlation between late loss and the need for repeat revascularization has been demonstrated recently.¹³¹

The classical binary definition of restenosis based on percentage diameter stenosis does not accurately depict the degree of deterioration of the vessel after angioplasty and does not convey a measure of the vessel’s response to injury. Angiographically detected lesions of $\geq 50\%$ diameter stenosis at follow-up have been historically considered as representing “restenosis”; however, binary restenosis erroneously assumes that a patient with 51% diameter stenosis and another with 49% diameter stenosis have different intimal hyperplasia responses.

Other angiographic parameters that may have reasonable correlation with clinical restenosis are MLD at follow-up and percentage diameter stenosis. The parameter percentage diameter stenosis carries with it the assumption of normal-appearing reference segments, which is known from IVUS studies to be an erroneous assumption.¹³² Furthermore, the location of the MLD also interferes with the calculation of reference vessel size and consequently affects percentage diameter stenosis.

Finally, the clinical surrogates of restenosis, target lesion and target vessel revascularization should be regarded as the true measure of DES treatment success; however, clinical end points are subjective and do not determine the biological antiproliferative efficacy of DES.

Mechanism of Restenosis after DES

Our understanding of the mechanisms of DES failure is still limited. It appears that the causes of restenosis after implan-



Figure 5. Cross-sectional IVUS image of black hole—with (left) and without (right) contours—after implantation of sirolimus-eluting stents in a restenotic lesion after brachytherapy.

tation of bare-metal stents and DES are fundamentally the same. The relative contributions of individual factors to the development of clinical restenosis, however, appear to differ considerably between DES and bare-metal stents.

The magnitude of the biological response of DES on neointimal proliferation has likely unmasked the contribution of 2 other aspects of restenosis after bare stents: (1) mechanical-related failures, including stent underexpansion,¹³³ strut fracture, and plaque prolapse, and (2) technique-related factors, including barotrauma outside the stented segment or uncovered atherosclerotic plaques (ie, geographical miss).¹³⁴ The US Cypher Post-Marketing Surveillance study included 2067 patients (3245 lesions) treated with at least 1 sirolimus-eluting stent in 38 US hospitals. Procedural data from 31 patients with restenosis of the target segment was evaluated by an independent core laboratory. Geographical miss, a technique-related phenomenon, was depicted in all 11 segments with edge restenosis (M.A.C., unpublished data, 2004). Stent strut displacement, which represents a mechanical failure, was noted in 1 patient. The S.T.L.L.R. (Prospective Evaluation of the Impact of Stent Deployment Technique on Clinical Outcomes of Patients Treated With the Cypher Sirolimus-Eluting Stent) study is prospectively evaluating the impact of deployment techniques in the outcomes of 1500 patients treated with sirolimus-eluting stents in multiple centers around the United States. Preliminary results showed an incidence of longitudinal geographical miss in 45.8% of the procedures.

Although DES have drastically reduced angiographic and clinical restenosis across broad lesion and patient subsets, certain anatomic and clinical scenarios, such as patients with diabetes mellitus, restenotic lesions after brachytherapy (P. Teirstein, MD, unpublished data, 2004) or DES,¹³⁵ bypass graft disease, and bifurcations, continue to be problematic. In addition, the characteristics of intimal hyperplasia may be altered by potent medications that interfere with cell division. Echolucent intimal tissue, termed black hole (Figure 5), also has been depicted by IVUS in patients with restenosis after DES implantation, particularly those who have previously failed brachytherapy (M.A.C., unpublished data, 2004). The molecular mechanisms involved in the development of black hole are not understood but likely represent an altered cellular response to vascular injury. The hypocellularity of this intraluminal tissue and the homogeneous distribution of proteoglycans may explain the lack of ultrasound signal (echolucent). Regions of acellular plasma-like collections

were recently observed at 30 and 90 days after DES implantation in porcine coronary arteries.¹³⁶ Future studies combining IVUS and histology are required to reconcile clinical IVUS findings with histological observations in experimental models. Difficulty in visualizing this echolucent tissue by IVUS may puzzle operators who encounter patients with angiographic restenosis that is “undetectable” by IVUS. In addition, this phenomenon may affect the proper quantification of intimal proliferation in DES trials. Further investigations are required to elucidate this novel biological vessel wall response and its association with potent antiproliferative agents.

The patterns of in-stent restenosis also have changed with DES and appear to be specific for each type of device. Restenosis after sirolimus-eluting stents are mostly (>90%) focal and usually located at the stent edges,^{137,138} whereas diffuse intimal proliferation or total occlusion accounts for ≈50% of the restenosis cases after polymer-coated Taxol-eluting stent implantation (A. Colombo, MD, personal communication, 2004). A tentative association between the mechanisms and patterns of restenosis is illustrated in Figure 6.

DES: Future Approaches

Endothelium and Restenosis

Endothelial integrity is essential for maintaining vascular homeostasis, and endothelial denudation results in neointimal thickening.^{139,140} Endothelial cell products inhibit platelet function and thrombosis, control vessel wall permeability,¹⁴¹ and bind and inactivate mitogens, thereby inhibiting SMC growth.¹⁴² Endothelial dysfunction may persist for >3 months after vascular injury and is more pronounced after bare-metal stent implantation as compared with balloon angioplasty.¹⁴³ Whether the development of a functional endothelial layer is further delayed after DES implantation remains to be investigated. Experimental evidence from animal models suggests that the antiproliferative properties of paclitaxel may limit endothelial cell regrowth.¹¹¹ This may represent the basis for the recommended prolonged (6-month-long) dual antiplatelet therapy for patients treated with paclitaxel-eluting stents. Endothelial cell recovery occurred to a similar extent (≈70%) in bare-metal stents and sirolimus-eluting stents implanted in dog coronary arteries.⁸⁴ Recent pathological analyses of human coronary arteries treated with sirolimus-eluting stents confirm almost complete reendothelialization of stents late after implantation.^{85,144} These results are reassuring, but the functional status of endothelial cells

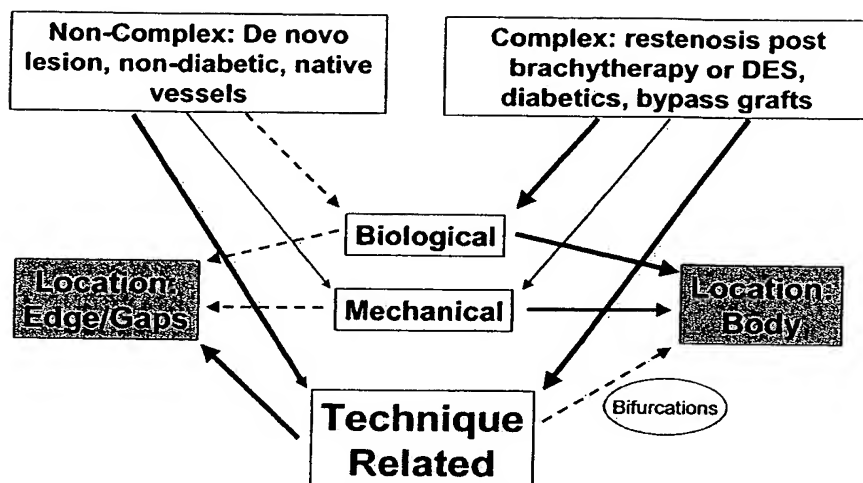


Figure 6. Tentative association between the mechanisms and patterns (location) of restenosis. Strong associations are represented by thick lines, whereas weak relationships are represented by dashed lines. Complex anatomic or clinical scenarios are associated with both biological- and technique-related failures. In noncomplex cases, the biological response is almost completely blocked by the antiproliferative agent. Restenosis in noncomplex cases are mainly associated with technique-related failures and appears mostly at the stent edges or gaps between stents. On the other hand, biological as well as mechanical failures are mainly associated with in-stent restenosis. Bifurcation lesion represents an exception to this general rule because it is usually associated with technique-related failures (ie, inability to effectively treat the ostium of the side branch) but may affect the body of the main branch stent.

covering DES was not assessed. Nevertheless, the incidence of stent thrombosis, which is one clinical consequence of abnormal healing and reendothelialization, remains low after DES implantation with recommended antiplatelet regimens.¹⁴⁵

Investigators have pursued diverse strategies, including pharmacological modulation, tissue engineering, gene and stem cell therapies, and even procedural modifications (ie, direct stenting) to limit endothelial injury, supplement endothelial cell products, accelerate endothelial cell regeneration, or all 3, to reduce neointimal thickening. Promoting rather than blocking the healing process by stimulating reendothelialization seems the most natural approach to prevent restenosis.

Rogers and coworkers¹⁴⁶ have explored whether stenting technique (ie, predilation versus direct stenting) and the degree of endothelial damage might determine later proliferative sequelae. *En face* staining of the luminal surfaces of stented iliac arteries of New Zealand White rabbits demonstrated endothelial cell loss immediately after stent expansion, which was restricted to interstices between stent struts. Remnant endothelium adjacent to struts provided the foundation for complete endothelial regeneration of the stented segment within 3 days. Vessel wall inflammation was reduced >80% in directly stented arteries, in concert with a 2-fold reduction in intimal thickening after 14 days, as compared with arteries completely denuded by balloon predilation before stent expansion. These data indicate that the degree of endothelial injury, which is influenced by stent and balloon design and deployment technique, is an important determinant in the biological repair response to vascular injury.

Seminal experiments from the laboratory of Edelman and associates using tissue-engineered perivascular endothelial cell implants have identified the heparan sulfate proteoglycan perlecan as a potent inhibitor of neointimal hyperplasia after deep vascular injury, largely as a consequence of its ability to modulate thrombosis and inhibit basic fibroblast growth factor binding and activity.¹⁴⁷ In these experiments, endothelial cells were seeded onto 3-dimensional polymeric matrices and implanted adjacent to porcine carotid arteries subjected to deep injury. Thus, in this model, the endothelial cells are far from the lumen, allowing their biochemical regulatory properties to be

dissociated from their boundary properties. Perivascular transplantation combined with the ability to genetically modify cultured endothelial cells provides a powerful tool to dissect the roles of various endothelial cell products in controlling the vascular response to injury. Although valuable from a proof-of-principle point of view, perivascular endothelial cell transplantation is obviously not practical clinically. Advances in tissue engineering may allow for endothelial cell seeding on stents.¹⁴⁸

Endothelial-derived NO also participates in the control of vascular healing by attenuating vascular inflammation and inhibiting SMC proliferation and migration.¹⁴⁹ With the highly efficient Sendai virus/liposome *in vivo* gene transfer technique, endothelial nitric oxide synthase (eNOS) gene transfection not only restored NO production of rat carotid arteries after endothelium denudation but also increased vascular reactivity of the injured vessels. Neointima formation at day 14 after balloon injury was inhibited by 70%. These observations provide further evidence that NO is an endogenous inhibitor of vascular lesion formation *in vivo* and suggest the possibility that strategies to increase eNOS or NO production are potential therapeutic approaches to treat neointimal hyperplasia.

Pharmacological strategies aiming at stimulating endothelial regeneration or NO production have been tested. Stents coated with oxygen free radical scavenger conjugated with polyesteramide coating were implanted in 45 patients in a multicenter study involving European and South American sites. The study failed to demonstrate the antirestenosis properties of NO donors. Estradiol has been shown to improve vascular healing, reduce SMC migration and proliferation, and promote local angiogenesis via NO-dependent and NO-independent mechanisms.¹⁵⁰ Stents eluting 17- β estradiol were implanted in porcine coronary arteries and reduced neointimal hyperplasia by 40% as compared with control stents.¹⁵¹ Estradiol-eluting stents were subsequently implanted in human coronary arteries in a single-center feasibility study involving 30 patients with *de novo* coronary lesions. The average concentration was 2.54 $\mu\text{g}/\text{mm}^2$ of stent. There were no deaths or stent thromboses, and 1 patient underwent target lesion revascularization up to the 12-month follow-up, but a moderate in-stent luminal loss was observed.¹⁵² Stents were loaded on site by immersion in a solution of

estradiol, which may have led to inadequate loading and elution of the drug.

Vascular endothelial growth factor (VEGF) has attracted attention for endothelial regeneration and angiogenesis.¹⁵³ VEGF is one of the most potent vascular permeability factors known and is thought to function as an endogenous regulator of endothelial integrity after injury, thereby protecting the artery from disease progression.¹⁵⁴ Previous animal studies have reported that local delivery of VEGF as naked DNA, adenovirus-mediated gene transfer, or recombinant protein after balloon- and stent-induced endothelial injury promotes endothelial regeneration, accelerates the recovery of endothelium-dependent relaxation, and reduces neointimal formation, suggesting the close correlation between accelerated endothelial integrity and reduced neointima after balloon and stent injury.¹⁵⁵⁻¹⁵⁹ There is still considerable debate, however, over the vasculoprotective versus atherogenic effects of VEGF.¹⁶⁰ Increased expression of VEGF and its 2 receptors, VEGFR-1 (*Flt-1*) and VEGFR-2 (*Flk-1*), has been reported in atherosclerotic and restenotic lesions.^{161,162} VEGF induces migration and activation of monocytes,¹⁶³ induces adhesion molecules¹⁶⁴ and MCP-1,¹⁶⁵ and enhances neointimal formation and atherogenesis by stimulating intraplaque angiogenesis in hypercholesterolemic animals without balloon injury^{166,167} or by increasing monocyte infiltration into atherosclerotic lesions.¹⁶⁸ Therefore, it has remained somewhat unclear whether VEGF protects the artery from vascular disease or accelerates vascular disease. A soluble form of the VEGF receptor-1 (*sFlt-1*) is expressed endogenously by vascular endothelial cells and can inhibit VEGF activity by directly sequestering VEGF and by functioning as a dominant-negative inhibitor against VEGF.¹⁶⁹ Blockade of VEGF by *sFlt-1* gene transfer attenuated neointimal formation after intraluminal injury in rabbits, rats, and mice.¹⁷⁰ *sFlt-1* gene transfer markedly attenuated the early vascular inflammation and proliferation and later neointimal formation. *sFlt-1* gene transfer also inhibited increased expression of inflammatory factors such as MCP-1 and VEGF. Thus, taken together, these observations suggest a far more complex story for VEGF in restenosis and indicate that increased expression and activity of endogenous VEGF are essential in the development of experimental restenosis after intraluminal injury by recruiting monocyte-lineage cells.

A phase II clinical study involving catheter-based local intracoronary gene transfer of VEGF (Kuopio Angiogenesis Trial) failed to reduce clinical and angiographic restenosis after balloon angioplasty and stenting.¹⁷¹ Recent exciting observations that VEGF-gene-eluting stents accelerate reendothelialization and reduce in-stent neointimal volume in animal models¹⁷² are likely to propel this approach to trials in humans, however.

The transplantation of endothelial progenitor cells (EPCs) represents a novel therapeutic approach to enhance endothelial cell regeneration, neovascularization, or both. EPCs are present in the systemic circulation and have been harvested from the peripheral circulation, expanded ex vivo, and administered to animals with limb or myocardial ischemia.^{173,174} Iwaguro et al¹⁷⁵

recently investigated ex vivo phenotypic modulation as a method to enhance EPC function and to reduce the number of EPCs required for transplantation. The number of EPCs required to observe these effects was 30 times less than that required in previous animal studies to improve ischemic limb salvage. The combination of gene therapy with the techniques of cell transplantation represents a potential method for accelerating endothelial regeneration.

Bone Marrow-Derived Cells

Although the role of bone marrow-derived cells in vascular repair and regeneration has not been well defined in humans, accumulating evidence suggests that somatic stem cells in the bone marrow are capable of differentiating into vascular endothelial cells^{176,177} and SMCs.^{178,179} After vascular injury, it is hypothesized that progenitor cells from bone marrow or blood compartments are mobilized by cytokine activation,¹⁸⁰ home to sites of vascular damage, proliferate, and form arterial lesions in conjunction with resident arterial cells. Evidence in support of these hypotheses comes from animal models of allograft vasculopathy and diet-induced atherosclerosis, in which bone marrow-derived cells may give rise to a substantial percentage of lesional vascular SMCs.¹⁸⁰⁻¹⁸²

Recent experimental findings from the laboratory of Nabel and colleagues strongly suggest that vascular repair and regeneration is regulated by the proliferation of bone marrow-derived hematopoietic and nonhematopoietic cells through a p27^{Kip1}-dependent mechanism and that immune cells largely mediate these effects.⁵⁴ Exploiting the power of genetically deficient mice and bone marrow transplantation, they reported that lesion formation after mechanical arterial injury was markedly increased in p27^{Kip1}-deficient mice, characterized by prominent vascular infiltration by immune and inflammatory cells. Vascular occlusion was substantially increased when bone marrow-derived cells from p27-deficient mice repopulated vascular lesions induced by mechanical injury in wild-type recipients, in contrast to wild-type bone marrow donors.

The contribution and importance of bone marrow-derived cells to human vascular disease is largely unproven. Histological analyses of vessels from sex-mismatched hearts after orthotopic cardiac transplantation have revealed that 10% of arterioles contained cells of host origin¹⁸³ and that 2.6% of the SMCs examined were host derived,¹⁸⁴ observations consistent with the migration of putative stem/progenitor cells from the recipient to the grafted heart. Similarly, human muscle cells of host origin were identified in the vascular lesions of renal allografts.¹⁸⁵

The use of antibodies against membrane receptors of circulating progenitor endothelial cells to attract these cells to the site of vascular injury has been proposed by Kutryk and coworkers. These investigators coated surface-modified stainless steel stents with anti-CD34 antibodies. They have found CD34⁺ cells covering 70% of the stent surface 1 hour after antibody-coated stent deployment in pig coronary arteries versus no cell coverage of stainless steel stents (M.J. Kutryk, MD, unpublished data, 2002). These antibody-coated stents have been implanted in human coronaries in a multicenter pilot study (Healthy Endothelial Accelerated Lining Inhibits Neointimal Growth [HEALING]) without adverse reactions, but long-term outcomes are pending.

Transcription Factor Decoy Oligonucleotides

Oligodeoxynucleotides (ODNs) bearing the consensus binding sequence of a specific transcription factor are capable of manipulating gene expression in living cells. This strategy involves the intracellular delivery of such "decoy" ODNs, which are then recognized and bound by the target factor. Occupation of the transcription factor's DNA-binding site by the decoy renders the protein incapable of subsequently binding to the promoter regions of target genes. The use of decoy ODNs for the therapeutic manipulation of gene expression was first described by Dzau's laboratory in 1995. Morishita et al¹⁸⁶ reported the treatment of rat carotid arteries at the time of balloon injury, with ODNs bearing the consensus binding site for the E2F family of transcription factors. Although as many as 6 E2F isoforms are known to play differing roles in cell cycle progression and cell growth,¹⁸⁷ release of the predominant E2F-1 isoform at a critical point in the late G₁ phase of the cell cycle coordinately upregulates expression of multiple genes that help speed the cell through DNA synthesis and mitosis. They found that a decoy specific to E2F-1 prevented this upregulation and blocked smooth muscle proliferation and neointimal hyperplasia in injured vessels.¹⁸⁶ On the basis of these preclinical data, these investigators examined the safety and biological efficacy of E2F decoy transfection in patients receiving human vein bypass grafts for peripheral vascular disease. Treatment with E2F decoy was associated with a >70% reduction in cellular proliferation markers, and at 12 months, fewer graft occlusions, revisions, or critical stenosis as compared with the untreated group (hazard ratio 0.34 [95% CI 0.12 to 0.99]). Application of this genetic-engineering strategy to PCI likely depends on the results of 2 large phase III trials designed to lower clinical failure rates after coronary artery bypass grafting and peripheral revascularization. There are significant challenges with ODN therapy because of issues of target specificity, uptake across cell membranes, and rapid intracellular degradation of the ODN. Indeed, the only randomized restenosis study in humans with antisense ODN directed against the nuclear protooncogene *c-myc* demonstrated no reduction in angiographic or clinical restenosis after bare-metal stenting.¹⁸⁸

Combination Chemotherapy

Combination chemotherapy—namely, the use of multiple agents to optimize efficacy and limit toxicity—is the principal treatment strategy in oncology. To date, single-drug approaches have dominated antirestenosis programs, but emerging experimental evidence indicates that combination therapies also may be effective in reducing neointimal thickening after stenting. A combination of hirudin and iloprost was blended with a polylactic acid polymer and loaded onto a stent. Although iloprost was slowly released by the breakdown of the polymer, ~60% of the hirudin was eluted in the first 24 hours.¹⁸⁹ Decreased neointimal formation was observed in sheep and pig injury models treated with this antithrombotic-eluting stent. Paclitaxel-NO donor conjugate-eluting stent was found to be more beneficial than was paclitaxel-eluting stent alone.¹⁹⁰ Gene therapy also may be combined with pharmacological therapy to modulate distinct

ligand-receptor signaling systems. Leppanen and coworkers¹⁹¹ recently reported that oral imatinib mesylate (STI571/gleevec) improves the efficacy of local intravascular VEGF-C gene transfer in reducing neointimal growth. Theoretical advantages of such combination approaches over single-drug antiproliferative therapy alone include larger reductions in neointimal growth by targeting multiple cell cycle checkpoints, diminished likelihood of resistance, and enhanced endothelial recovery.

Customized stents for drug delivery have been developed. These stents may allow both temporal and spatial control of drug release. Thus, an antithrombotic, prohealing agent could be released in the luminal surface early after implantation, whereas an antiproliferative agent could be released intramurally. The Conor stent¹⁹² has individual polymer inlays for drug reservoirs that can be loaded with different compounds.

Biodegradable Stents

Polymeric biodegradable stents, which "dissolve" slowly after implantation, are promising stent technologies that can be loaded with large amounts of drug or multiple agents. Biodegradable stents fulfill the ideal requirement for endovascular prosthesis by providing initial scaffolding support to prevent vessel recoil and negative remodeling, without the undesirable continuous vessel trauma caused by a permanent rigid foreign body. Still, polymeric stents have yet to achieve the mechanical strength or surface properties of stainless steel stents. In addition, vessel toxicity remains a major limitation for polymeric biodegradable stents.¹⁹³ The Igaki-Tamai stents have been tested in human coronary arteries with satisfactory results,¹⁹⁴ but stents were still visible by IVUS 6 months after implantation. Stents made of magnesium alloys have been developed recently. Biocorrosion of magnesium AE21 alloy containing 2% aluminum atoms and 1% rare earth elements (cerium, praseodymium, neodymium) was well tolerated in pig coronary arteries.¹⁹⁵ Inflammatory reaction, albeit not extensive, was induced by corrosion of the stent, which lost its integrity ~35 days after implantation. Positive vessel remodeling was observed after this period, which may be related to the inflammatory reaction. The ability of this metallic stent to carry medication, with or without a biodegradable polymer coating, remains to be demonstrated. Further experiments are required to exclude potential vessel toxicity and hydrogen formation that may be associated with the corrosion of magnesium.

Conclusion

The elucidation of the molecular and cellular mechanisms of inflammation and cellular proliferation in vascular injury and repair powered the development of DES technology. Despite across-the-board benefits with DES as compared with bare-metal stents in randomized clinical trials and registries, significant challenges remain. Advances in drugs and devices are needed for the treatment of small vessels, left main and bifurcation lesions, and patients with diabetes mellitus. Combination chemotherapy, biodegradable stents, and cell-based therapies are likely to provide effective solutions for the prevention of restenosis in complex lesions and patients.

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